GENETIC DISTANCE IN THE GENUS *EPHIPPIGER* (ORTHOPTERA, TETTIGONIOIDEA) - A RECONNAISSANCE

by

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Abstract

Oudman, L., W. Landman & M. Duijm, 1989. Genetic distance in the genus *Ephippiger* (Orthoptera, Tettigonioidea). – a reconnaissance. – Tijdschrift voor Entomologie 132: 177-181, figs 1-2, tabs 1-3. [ISSN 0040-7496]. Published 1 December 1989.

Genetic distances were determined by means of enzyme electrophoresis for a number of *Ephippiger* (sub)species, mainly from southern France and northern Italy. For each (sub)species and form one 'typical' location was selected. The results are summarized in a dendrogram. The three groups distinguished by Duijm & Oudman (1983) on the base of copulatory behaviour and morphological characters are confirmed. Nei's genetic distances between *E. ephippiger*, *E. cruciger* and *E. cunii* appeared to be low for genuine species. A comparison with *Uromenus rugosicollis* is included.

Keywords. – *Ephippiger*, enzyme electrophoresis, genetic distance, dendrogram.

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Introduction

In France, the northern part of Spain and NW Italy a number of *Ephippiger* (sub)species occur (e.g. Chopard 1951; Harz 1969). For this region Duijm & Oudman (1983) recognised three groups of (sub)spieces, viz. 1 – *Ephippiger provincialis* (Yersin, 1854), 2 – *E. terrestris* (Yersin, 1854) with the three (sub)species *E. t. terrestris*, *E. t. hormansi* (Brunner von Wattenwyl, 1882) and *E. t. caprai* Nadig, 1980, 3 – *E. ephippiger* (Fieber, 1853) (the subspieces *E. e. vitium* (Serville, 1831)[1]) and *E. e. vicheti* Harz, 1966) *E. cunii* (Bolivar, 1877) and *E. cruciger* (Fieber, 1853). Between these groups no mating is possible. Within group 2 no mating barriers were found, whereas mating between species of group 3 is possible (Hartley & Warne 1984), but not in all cases (Duijm & Oudman 1983). Identification of single specimens and even populations belonging to group 3 often meets with considerable difficulties owing to the large variability of the morphological characters. The present study is an attempt to elucidate the relations between these *Ephippiger* taxa by the investigation of enzyme polymorphism. For comparison the Ephippigerid *Uromenus rugosicollis* (Serville, 1839) is used.

Materials and methods

The insects were collected during field trips in August and September of 1979, 1980, 1981, 1982 and 1983. Generally we succeeded in collecting a sufficient number (*c* 20) from a restricted area of a few acres. This area had to be small to limit ourselves to one population (or part of it) and so to avoid the mixing of different populations. The animals were killed, measured, photographed and frozen in solid carbon dioxide (-79°C).

For this "reconnaissance" it appeared desirable to omit the intra- (sub)specifical variation in order to get a clearer picture. For each taxon we therefore selected one locality that we considered sufficiently typical. In this selection we used – if possible – the type locality or our nearest collecting site and otherwise a locality that was in good concordance with the morphological description and/or in the neighbourhood of the centre of distribution[2]). The

[1]) According to Kruseman (1988) the correct name for this subspecies is *E. ephippiger diurnus* Dufour, 1841.

[2]) Later work (Landman et al. 1989) showed that the population used in this study as representative for *E. t. terrestris* (Col de Castillon, No. 2), though in many respects very close to the nominate form cannot be regarded as entirely "pure".

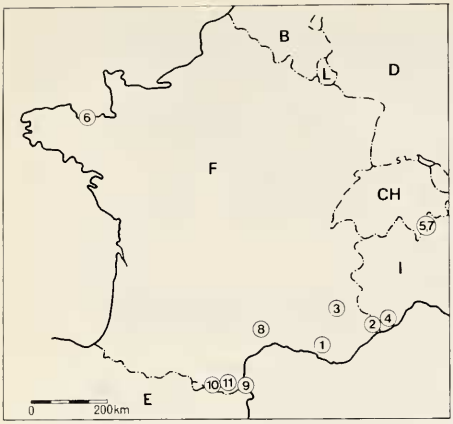

Fig. 1. Collection sites of *Ephippiger* and *Uromenus* species in SW Europe. Numbers denote the sites, see table 1.

sites selected are summarised in table 1 and fig. 1.

For preparation of the samples, electrophoretic techniques and the preparation of the horizontal polyacrylamide gels we refer to Van Dijk & Van Delden (1981).

The following loci were analysed: Alcohol dehydrogenase (Adh), Tetrazolium oxidase (To), two Phosphoglucomutases (Pgm-2 and Pgm-3), Malic enzyme (Me), Fructosediphosphate aldolase (Ald), Esterase-2 (Est-2), Hexokinase-3 (Hk-3), Fumarate hydratase (Fum), Xanthine dehydrogenase (Xdh), Isocitrate dehydrogenase (Idh), Glucose oxidase-3 (Gluo-3), α-Glycerophosphate dehydrogenase (α-Gpdh). For polymorphic loci Mendelian inheritance was assumed on base of enzyme band patterns and checked by testing genotype frequencies for every location for Hardy-Weinberg equilibrium.

Between populations Nei's genetic distances were calculated (Nei 1975). From the matrix of

Table 1. Sites of collection of the selected populations of *Ephippiger* and *Uromenus* species.

No.	(Sub)species	Location	Country/dept.	Alt. m.	Date
1.	*E. provincialis*	Plan d'Aups	F 84	680	24-viii-80
2.	*E.t. terrestris*	Col de Castillon	F 06	700	31-viii-81
3.	*E.t. terr.* f *minor*	Col de Maure	F 04	1350	24-viii-81
4.	*E.t. caprai*	Cle. Scravaion	I Liguria	820	3-ix-81
5.	*E.t. bormansi*	Naggio (L. di Como)	I Lombardia	800	5/6-ix-81
6.	*E.e. diurnus*	Cap Fréhel	F 22	70	9-ix-83
7.	*E.e. vicheti*	Naggio (L. di Como)	I Lombardia	750	5/6-ix-81
8.	*E. cruciger*	Gignac	F 34	60	3-vii-82
9.	*E. cunii*	Cerbère	F 66	10	9-vii-82
10.	*E. cunii* f. *jugicola*	Val d'Eyne	F 66	1600	22-viii-82
11.	*Uromenus rugosicollis*	Canigou	F 66	840	31-viii-79

Table 2. Allele frequencies of the polymorphic loci of *Ephippiger* and *Uromenus* species.

	(sub)species	n	Pgm-2				Pgm-3			
			12	14	16	20	26	29	32	35
1	*E. provincialis*	16	0	0	0	1.00	0	.14	.86	0
2	*E.t. terrestris*	18	.50	.50	0	0	.27	.67	0	.07
3	*E.t.t.*f. *minor*	13	.23	.77	0	0	0	0	.69	.31
4	*E.t. caprai*	23	.28	.65	.07	0	0	.96	.04	0
5	*E.t. bormansi*	18	.69	.31	0	0	0	0	1.00	0
6	*E.e. diurnus*	20	0	0	0	1.00	.70	.30	0	0
7	*E.e. vicheti*	21	0	0	0	1.00	1.00	0	0	0
8	*E. cruciger*	26	0	0	0	1.00	.35	.23	.31	.11
9	*E. cunii*	20	0	0	0	1.00	.20	.80	0	0
10	*E. cunii* f. *jugicola*	24	0	0	0	1.00	0	1.00	0	0
11	*Uromenus rugosicollis*	15	–	–	–	–	.13	.53	.33	0

genetic distances a dendrogram was constructed following the UPGMA method (Sneath and Sokal 1973).

Samples of collected (sub)spieces from all localities, including tips of abdomens used for electrophoresis, will be deposited in the Entomological collection of the Institute for Taxonomical Zoology (Zoological Museum) in Amsterdam.

RESULTS

Thirteen loci were investigated of which eight were monomorphic. Five loci showed polymorphism: Est-2, Pgm-2, Pgm-3, To and Adh, with respectively 4, 4, 4, 3 and 2 alleles. The allozyme frequencies are given in table 2. To is fixed in most populations. Adh is only polymorphic in *E. t. caprai*. Pgm-2 is only variable in *E. terrestris*. The most variable enzymes are Pgm-3 and Est-2.

The genetic distances are shown in table 3 and the dendrogram, calculated from these distances, in fig. 2.

Based on a preliminary investigation (Landman, 1981) we determined the genetic distance between *Uromenus rugosicollis* and a number of *Ephippiger* (sub)spieces (18 populations, 9 loci, 18 alleles) at 0.3473. An indication of this distance is added to the dendrogram.

DISCUSSION

The genetic distance (0.35) between the closely related genera *Uromenus* and *Ephippiger* appears to be very low in view of the range for genera mentioned in reviews (e. g. Thorpe 1982, Menken & Ulenberg 1987). The distance between genera generally is 1, in the mean 1.30, and minimally 0.62.

The distance found by us between *E. provincialis* and the other *Ephippiger* species (0.20) as well as the distance between our groups 2 and 3 (0.16) are very low for congeneric species. According to Thorpe (1982) only in 3% of the cases studied distances below 0.16 are found for congeneric species.

The distances between the subspecies of *E. terrestris* (0.015 – 0.11) are within the range generally found between subspecies: 0.02 – 0.22 (Menken & Ulenberg 1987) except one: the distance (0.015) between *E.t. caprai* and *E.t. terrestris* from Col de Castillon. This very small distance is one of the indications that the population of Castillon is not quite representative for the nominate form of *E. t. terrestris*.

The distances between the species within our group 3 are strikingly small (0.03); they lie in the range for subspecies. This is in accordance with the results of Hartley and Warne (1984).

The dendrogram of fig. 2 offers a picture that is mainly in accordance with current taxonomical opinion. It also conforms to our grouping (Duijm & Oudman 1983) based on morphological data and on the existence of mating barriers. Within group 3, however, *E. e. vicheti* occupies a rather separate position [1]). The relations between *E. e. diurnus*, *E. cruciger* and *E. cunii* as well as those within the *terrestris*-group will be dealt with in later publications.

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[1]) Nadig (1987,p.331) raises this taxon to species-level: *E. vicheti* Harz, 1966.

(Table 2 continued)

Est-2				To				Adh	
15	17	20	23	40	63	65	67	18	22
.78	.22	0	0	0	1.00	0	0	1.00	0
.08	.67	.25	0	0	0	0	1.00	1.00	0
.42	.23	.35	0	0	0	0	1.00	1.00	0
.10	.57	.33	0	0	0	0	1.00	.80	.20
0	.24	.76	0	0	0	0	1.00	1.00	0
0	1.00	0	0	0	0	0	1.00	1.00	0
0	0	.08	.92	0	0	0	1.00	1.00	0
.04	.50	.46	0	.06	0	0	.94	1.00	0
.25	.67	.08	0	0	0	0	1.00	1.00	0
.08	.33	.54	.04	0	0	0	1.00	1.00	0
0	.02	.90	.08	0	0	1.00	0	1.00	0

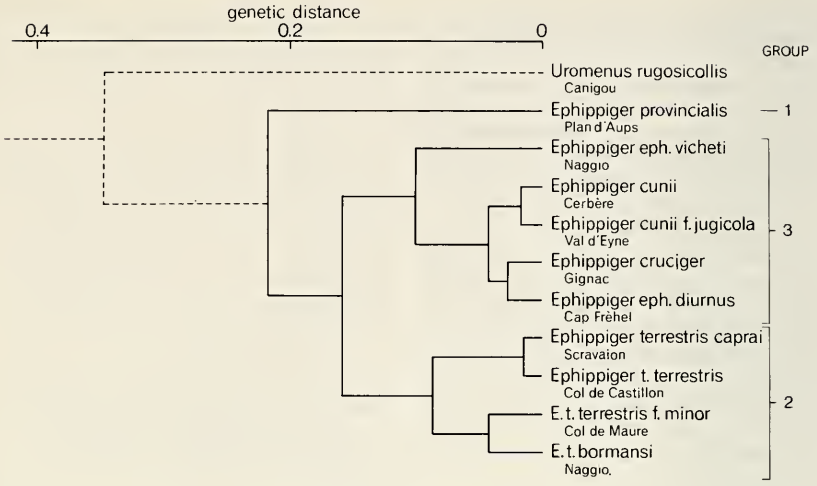

Fig. 2. Dendrogram of Ephippigeridae based on the genetic distances of table 3. An estimation of the distance between *Uromenus* and the *Ephippiger* (sub)species is added on the base of preliminary work (Landman, not published).

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Table 3. Genetic distances based on allozyme frequencies of thirteen loci of *Ephippiger* species.

Group		(sub)species	1	2	3	4	5	6	7	8	9	10
1	1	*E. provincialis*	–	.2522	.1840	.2668	.2167	.1971	.2337	.1408	.1631	.1890
2	2	*E.t. terrestris*		–	.0680	.0147	.0899	.0858	.1687	.0846	.0687	.0815
	3	*E.t.t.* f. *minor*			–	.0792	.0409	.1649	.1956	.1016	.1439	.1514
	4	*E.t. caprai*				–	.1115	.1232	.2152	.1073	.0761	.0726
	5	*E.t. bormansi*					–	.1908	.2192	.1059	.1771	.1598
3	6	*E.e. diurnus*						–	.0832	.0288	.0276	.0717
	7	*E.e. vicheti*							–	.0766	.1109	.1309
	8	*E. cruciger*								–	.0287	.0363
	9	*E. cunii*									–	.0179
	10	*E. cunii* f. *jugicola*										–

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